GROWTH OF THE SOUTH AFRICAN ABALONE (*HALIOTIS MIDAE*) ON THREE DIETS, UNDER COMMERCIAL CONDITIONS.

by

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ABSTRACT

Haliotis midae is the cornerstone of the South African abalone fishery. For more than a decade, the wild abalone stock of South Africa has suffered decline due to over-exploitation and illegal activities such as poaching. Prior to 1970, no regulations were in place concerning the annual landings. As a result the fishery was exploited as if it were an infinite resource. It is this initial uncontrolled harvesting (regardless of age) and poaching that has driven the abalone resource decline. Due to the slow growth rate exhibited by abalone as a species, natural replenishment of wild stock following exploitation and poaching was far below the rate of exploitation of this resource.

Studies on the growth of abalone have mainly been conducted under laboratory conditions. The purpose of this study was to measure the growth of abalone, fed different diets, under commercial culture conditions. Three food types were used namely; commercial pellets, seaweed (*Ulva spp*.) and dried kelp bars (*Ecklonia maxima*). Four diets were obtained from the three food types namely; combination of commercial pellets and seaweed (Diet A), commercial pellets only (Diet B), seaweed only (Diet C) and dried kelp bars only (Diet D). The food types used in this study represent both artificial (Commercial pellets) and natural feeds (seaweed and kelp) used in commercial abalone culture.

The growth of two cohorts (40-50 mm and 50-60 mm) was followed over a 426 day period, with data for the first 183 days being used for statistical analysis to determine performance of a given diet.

The best growth rates were found in abalone fed Diet A (40-50 mm: 2.64 mm.month^{-1}; 50-60 mm 2.78: mm.month^{-1}) and B (40-50 mm: 2.20 mm.month^{-1}; 50-60 mm: 2.35: mm.month^{-1}). These (Diets A and B) gave higher growth rates when compared to Diets C and D (natural diets), whose growth rates ranged between 0.50 mm.month^{-1} and 1.71 mm.month^{-1} for both cohorts. Also observed in this study was that, the mixture of formulated diet and seaweed gave better growth than formulated diet given exclusively.

Key words: *Haliotis midae*, Diet, Growth, Kelp, *Ulva spp*.

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Dedicated to my Parents

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Chapter 1

INTRODUCTION

1.0 Introduction

Abalone are gastropods belonging to the family Haliotidae (Barkai and Griffiths, 1986; Dlaza, 2006) and sub class Archaeogastropoda (Barkai and Griffiths, 1986; Sales & Britz, 2001). They are classified under a single genus of approximately more than 90 marine species (Sales & Britz, 2001), widely distributed from cool temperate to tropical regions. Haliotids are herbivorous deposit scrapers inhabiting shallow coastal areas world wide (Najmudeen & Victor, 2004) with rocky shores, between the intertidal and littoral zone (Hecht, 1994) where they lead a cryptic life as juveniles and aggregate as adults in shallow rocky waters (Werner *et al*., 1995; Muller, 1986). Abalone mainly feed on seaweed (McShane and Smith, 1988), although food preferences differ among species depending on habitat and food availability (Barkai and Griffiths, 1986; Dunstan *et al*., 1996).

The worldwide demand for this valuable shellfish species has exceeded its supply (Werner *et al*., 1995) resulting in increased fishing pressure on the fishery which in turn has led to a decline in wild stocks globally (Gordon & Cook, 2004; Shepherd *et al*, 1991). In some cases such as in California, the possible extinction of at least a single species; *Haliotis sorenseni* (Davis *et al*., 1992; Poore 1972) has been reported. In the case of California, the reasons for this decline and eventual collapse is not clear, being attributed to a combination of

factors such as over- fishing, pollution and disease as the most likely causes (Gary 1995).

Shepherd *et al*. (1991) attributes the global decline of abalone wild stock to the removal of great numbers of parental stock which today is evident in the rate of recruitment, as a result of exploitation and poaching. The abalone fishery has faced and continues to face four forms of exploitation, i.e.; recreational, subsistence, commercial and poaching (Hauck and Sweijd, 1999).

1.1 Aquaculture

The decreasing commercial catch and the high market demand for abalone in both the domestic and export markets (Gordon & Cook, 2004;Bautista- Teruel & Millamena, 1999; Bautista- Teruel *et al*. 2003; de Waal *et al*. 2003) coupled with over-exploitation of wild abalone stocks through poaching, led to the development of intensive shore-based abalone aquaculture in several countries including USA, Mexico, South Africa, Australia, New Zealand, Japan, China, Taiwan, Ireland, and Iceland (Hahn, 1989; Gordon and Cook, 2001). The most developed industry being is Japan, Taiwan and the USA (Hahn, 1989). Aquaculture has origins in Asia with China being the initial main contributor (Hecht and Britz, 1990). However, the fast growth in abalone aquaculture has mainly been due to the lucrative market value attached to the muscular foot and shell of this shellfish species (Oakes & Ponte, 1996). Despite the slow growth and resulting long culture periods of abalone, mariculture has become a viable supplier for the growing market demand of abalone worldwide.

Gordon and Cook (2004) showed that mariculture plays a vital role in the supply of abalone, with global production reaching 22,600 metric tonnes (including poaching of 3700 metric tonnes) in 2002. Of this, 8600 metric tonnes were farmed. China is the largest producer in the world with over 300 farms and a total production of approximately 4500 metric tonnes (Gordon and Cook, 2004). Outside of Asia, South Africa stands out as the largest abalone producer (Gordon and Cook, 2004). Favourable coastal water quality and infrastructure has facilitated rapid growth of the abalone industry in South Africa (Gordon and Cook, 2004). However, access to suitable coastal land and the dependence on wild harvest of kelp for feed (logistics and supply) may restrict further development in certain areas (Troell *et al*., 2006; Gordon and Cook, 2004). Other factors that influence success in aquaculture globally include water flow (Kautsky and Floke, 1989; Capinpin Jr. *et al*, 1999), stocking density (Huchette *et al*, 2003; Mgaya and Mercer, 1995), water temperature (Steinarsson & Imsland, 2003) and food availability i.e., food quality and quantity (Bautista- Teruel *et al*, 2003; Capinpin Jr. & Corre, 1996).

1.2 *Haliotis midae*

Six species of abalone are known to occur in the coastal waters of South Africa. These are *Haliotis midae*, *H. spadicea*, *H. parva*, *H. speciosa*, *H. queketti* and *H. pustulata* (Muller, 1985). Of the six, only *Haliotis midae* occurs in sufficiently large numbers to warrant commercial exploitation (Cook, 1998; Muller, 1986).

Haliotis midae is also the largest of the six abalone species found in South Africa, growing to approximately 230 mm shell length (Muller, 1986). Tarr (1995) puts

the maximum shell size at 200 mm at an age of 30 years. *Haliotis midae* is the cornerstone of the South African abalone industry (Newman 1967), having a fairly good distribution with the bulk of its commercial catch being made between Cape point and Cape Agulhas in comparison to its distribution between St. Helena Bay on the west coast and southern Transkei on the east (Barkai & Griffiths 1987). The bulk of the *H. midae* market is in Asia where it is served in different ways (Sales & Britz, 2001), with cocktail abalones (40-70 mm shell length) being in great demand in this part of the world (Jarayabhand & Paphavasit, 1996; Cook, 1991).

The variation in the west to east coastal water temperatures gives uniqueness to *Haliotis midae* with regard to habitat, with cooler temperatures to the west due to Benguela upwelling currents and warmer to the East as a result of the Agulhas currents (Newman, 1967; Britz *et al*., 1997). Newman (1967), Tarr (1995) and Wood (1993) argue that temperature has an inverse relationship to size of animal (age at sexual maturity) and growth rate. In South Africa this phenomenon is observed with the Eastern Cape population maturing at a smaller size in comparison to the Western Cape population (Britz *et al*., 1997). Hecht and Britz (1990), argue that this variation in shell size could be as a result of relatively higher primary production, higher food availability and oxygen in cooler waters of the West compared to the warmer Eastern Cape waters.

1.3 *Haliotis midae* fishery

The commercial exploitation of *Haliotis midae* has been going on since 1950 along the south and south-west coast of South Africa (Newman 1967). As in

other countries where abalone is exploited commercially, *H. midae* showed a decline in numbers collected (Tarr, 2000). Regulations of the fishery in South Africa were introduced in 1970. Prior to this no limits on harvest either by size or quantity was in place (Hauck and Sweijd, 1999). It is this decline that prompted the division of Sea Fisheries to initiate an abalone research program encompassing the biology of this species, so as to best manage the available natural stock (Newman, 1966).

The reason for the decline of the abalone fishery in South Africa for the past two decades is attributed to social (Hauck and Sweijd, 1999) and ecological changes (Tarr *et al*., 1996; Cook, 1998). Socially, an unexpected increase in poaching since 1994 altered the decline in the fishery for worse (Tarr, 2000), while ecologically the disappearance of the sea urchin (*Parechinus angulosus*) following the movement of the rock lobster (*Jasus lalandii*) into the kelp beds is believed to have had an effect on the *H. midae* stocks (Tarr, 2000). The sea urchins provide juvenile abalone with protection from predation (Day & Branch, 2002; Tarr, 2000). Juvenile abalone find refuge beneath the sea urchins (Day & Branch, 2000a; Tarr *et al*., 1996). Therefore, direct predation and reduction of sea urchin populations had an impact on juvenile abalone abundance in areas where their natural shelters (boulders) and refuge such as the sea urchin were absent. This associational behaviour is thought to be primarily one of protection for the abalone juveniles. Day and Branch (2000b) argue that abalone benefit in terms of diet from the drifting seaweed trapped by the sea urchins.

The total allowable catch (TAC) for the fishing season 1990/91 was 595 tons, and within a period of 7 years (1997/98 season) had dropped to 530 tons (South African commercial review). Reports for the 2006/07 TAC indicate a further decline to 125 tons (The Herald 26 October 2007). The reason for the continued decline has been attributed to poaching and movement of the rock lobster into perlemoen areas. As a result the government of South Africa through the ministry of Environmental Affairs and Tourism has proposed to put in place a ban on perlemoen harvesting by February 1 2008 (The Herald, 1 November 2007) so as to reduce the decline of this resource.

1.4 Factors influencing abalone development

1.4.1 Habitat and behaviour

Space is a potentially limiting resource for sessile invertebrates (Roughgarden *et al*., 1985) although one cannot consider it in isolation from biotic factors such as predation (Connell, 1985). In the absence of biotic factors such as predation, as in a culture system, space is an important aspect affecting growth in abalone. Solid surface is a preferred substratum for abalone. As a result open areas of bare sand are considered to pose an effective barrier to abalone movement (de Waal *et al*. 2003). This and other factors such as temperature (Steinarsson & Imsland, 2003) and food availability (Steinarsson & Imsland, 2003) may explain the discontinuous distribution of many of the haliotid populations globally.

Many abalone species aggregate (Hines and Pearse 1982; Prince 1992; Shepherd and Partington 1995), possibly to enhance fertilization (Uki & Kikuchi 1984; Hahn 1989a, pp 53-70), refuge (Hines and Pearse 1982; Shepherd 1986b)

or to trap drifting food of eat what others drop (Shepherd, 1973; McShane *et al*. 1988a).

Haliotis midae like other haliotid species is a broadcast dioecious spawner (Newman, 1967) depending on proximity of con-specifics for successful reproduction (Hahn 1989a, pp 53-70).

1.4.2 Temperature

Water temperature is considered the main exogenous factor, regulating the reproductive cycle in abalone (Hahn 1989) as it influences both the maximum size and the growth rate (Newman 1967). Temperature plays an important role in the metabolism and energy expenditure of poikilotherms (Britz *et al*., 1997). Therefore, understanding its effect on the physiology of abalone is important for management of both fisheries and aquaculture (Britz *et al*., 1997). Temperature maintains a direct relationship with growth rate (Hahn, 1989b, pp. 135-156) and other whole-body functions involved with energy metabolism, i.e. respiration (Barkai and Griffiths, 1987), food consumption (Preece & Mladenov, 1999; Hahn, 1989b, pp. 135-156), and excretion (Barkai and Griffiths, 1987) in invertebrates. Due to haliotids being thermoconformers, (Garcia-Esquivel *et al*, 2007; Prosser, 1991) whose tolerance range and optimal temperature conditions for feeding and growth are species-specific and depend on evolutionary adaptations (García-Esquivel *et al*., 2007), research towards diet development is therefore specific to a given species.

1.4.3 Diet

Diet preference has been observed in haliotids with Australian abalone showing preference for red algae over brown algae (Shepherd, 1973; Wells and Keesing, 1989) whereas the reverse has been suggested for abalone from North America (Leighton and Boolotian, 1963), South Africa (Barkai and Griffiths, 1986) and Japan (Uki *et al*., 1986). One of the hypothesized reasons for this preference is the presence of unpalatable chemicals which act as deterrents against herbivory (Steinberg, 1988; Shepherd and Steinberg, 1992). Fleming (1995) argues that the reason why some diets appeal to the abalone is because of presence of attractants in the diet.

Benthic diatoms are considered the principal food for post-larval and juvenile abalone that are unable to consume macroalgae (Kawamura *et al*. 1998; Gordon *et al*. 2006). Commercial abalone hatcheries use plastic plates covered with crustose red algae for settling larvae (de Waal *et al*, 2003; Dustan *et al*, 1996) and a film of diatoms as a food source for growing the post larvae (Hahn, 1989; Takami *et al*., 1997; Kawamura *et al*., 1998; Padermsak & Nittharatana, 1996; Fleming *et al*. 1996; Knauer *et al*. 1996), which are weaned on to macro algae (Dustan *et al*. 1996; Najmudeen & Victor, 2004) or artificial feed exclusively at a size of 3-7 mm shell length (Guofan *et al*. 2004).

Abalone culture today is becoming reliant on formulated diets (Viana *et al*, 1993; Serviere-Zaragoza *et al*, 2001; Kruatrachue *et al*, 2004 Britz *et al*., 1994). Artificial diets have been tested and shown to improve the growth rates of juvenile and young adult abalone in comparison with their natural diet (Uki *et al*.,

1985; Nie *et al*., 1986; Hahn, 1989; Uki and Watanabe, 1992; Viana *et al*., 1993; Viana *et al*., 1996; Mia *et al*., 1994 and Mia *et al*., 1995; Evans & Langdon 2000). Broader and Shpigel (2001) noted that feeding adult abalone *Haliotis roei* with enriched seaweed with a higher protein content than wild seaweed yielded growth rates similar to that achieved with formulated diets.

Formulated feeds of abalone must contain sufficient protein, carbohydrates, lipids and essential amino acids as adequate nutritional requirements (Fleming *et al*, 1996; Durazo-Beltran *et al*, 2003). The most common protein sources are: fishmeal, defatted soyabean meal and casein (Guzman and Viana, 1998). Fishmeal dried at a low temperature (70-80 ° C) by means of indirect heat has been efficiently utilized by *Haliotis midae* (Britz, 1994; Britz, 1996b). Of the three protein sources, only fish meal can support good growth performance, unlike soybean meal and casein which are fed in combination with other protein sources (Fleming *et al*, 1996).

In the study by Britz *et al.* (1994) and Sales & Britz (2001) it was suggested that Spirulina could have a very good potential as a protein source in *Haliotis midae* diets. Britz (1996a) observed a significant increase in growth rate with increasing dietary protein levels for *Haliotis midae*. Similar results have been documented for other haliotid species, such as in the study by Guzman & Viana (1998).The higher growth in many of these studies has been attributed to a higher protein content and quality in the feed (Viana *et al*., 1993). In order to achieve maximum growth, the rate of protein deposition by abalone must be maximized. This

implies that formulated feeds should contain sufficient protein (Britz and Hecht, 1997), which also must be readily digestible, having essential amino acids in correct proportions to each other. The amount of artificial dry feed ingested by abalone appears to be governed by their metabolic rate; as consumption in *Haliotis discus hannai* (Hahn, 1989) and *Haliotis midae* (Britz *et al*., 1997) was shown to be a predictable function of body size and temperature.

Feed costs make up a major portion of operating costs for any form of intensive aquaculture (Britz *et al*., 1997). Accurate estimation of daily intake to reduce over feeding which would result in wastage and poor water quality, or underfeeding, resulting in suppressed growth, are import to aquaculturalists (Britz *et al*., 1997).

Due to the relatively high cost of protein- based formulated diets such as casein (Guzman & Viana, 1998) and fishmeal (Britz *et al*. 1997), their usefulness as abalone feeds requires further study. Artificial diets based on less expensive protein sources are becoming important as an alternative to live natural feeds in the aquaculture industry (Shipton & Britz, 2001; Britz, 1996b). Therefore the response of abalone to formulated diets is vital to producing an effective low-cost diet for the animal. Kawamura *et al*., (1998) argue that poor and unpredictable growth performance is a function of variability in food, species of abalone and the growing conditions in hatcheries.

The abalone is an animal with slow and very heterogeneous growth (Viana *et al*., 1993; Fleming *et al*, 1996; Bautista *et al*, 2003).

Hahn (1989) places growth rate at approximately 2-3 cm per year. It is the slow rate of growth that has motivated the need for research into proper nutrition that allows for successful and faster culture of abalone (Viana *et al*., 1993; Fleming *et al*, 1996). The future of abalone aquaculture depends on good growth rates (Lopez *et al*., 1998). Hahn (1989) argued that factors such as quality of diet, feed intake and water temperature play a vital role in feed conversion ratios (FCR), which determine abalone growth rate under culture. Britz *et al*. (1997) observed that growth rate of *Haliotis midae*, fed artificial diets at varied water temperatures, showed a significant increase with temperature up to 20°C. Interspecific competition has been observed to have an effect on growth rate as a result of stocking density (Barki *et al*., 2001). Depending on growth rate, faster grows shall out perform slow growers hence the effect seen with growth rate variation.

1.4.4 Density

Most of the research on density-dependent growth in abalone has been restricted to aquaculture experiments (Mgaya and Mercer, 1995; Hunt *et al*, 1995; Huchette *et al*., 2003) the outcome of which was a negative correlation between growth rate and density. Other than density, ammonia is believed to influence growth and survival in abalone mariculture. Various studies have investigated the influence of ammonia on the survival and growth of abalone (Harris *et al*., 1998; Basuyaux and Mathieu, 1999; Hindrum *et al*, 2001; Huchette *et al*., 2003). Ammonia has been shown to affect the immune response such as in the Taiwanese abalone *Haliotis diversicolor supertexta* (Reddy-Lopata *et al*. 2006) and kidney structure in green lip abalone *Haliotis laevigata* (Harris *et al*. 1998),

ultimately influencing growth. There is little information available to South African abalone farmers on aspects such as ammonia toxicity and the influence of ammonia on growth of the locally farmed species *H. midae* (Reddy-Lopata *et al*. 2006).

1.5 Aim of Study

This study aimed at determining the growth rate for *Haliotis midae* fed three food types (commercial pellets, seaweed (*Ulva spp*.) and dried kelp bars (*Ecklonia maxima*), under commercial conditions. The objective of which is to obtain results that have a direct bearing on the commercial operations at Marine Growers and possibly other abalone farms within South Africa.

Chapter 2

MATERIALS AND METHODS

2.1 Sorting and setup

The research was conducted on the Marine Growers (South 33° 46.4', East 23° 43.2') abalone farm at Hougham Park. Juvenile *Haliotis midae* were sorted into two size classes by Marine Growers' management; 40-50 mm and 50-60 mm shell length. Prior to sorting, the animals were anesthetised using magnesium sulphate at 7% concentration, so as to reduce fatalities caused by handling stress. In order to place the abalone into a size class, a pilot cohort was measured using Vernier callipers after which visual observation was used to place the remaining abalone into their respective class size.

Approximately 250 animals were sorted into each of the 36 baskets (66 x 50 x 46 cm) assigned to the 40-50 mm class size and 150 animals into each of the 36 baskets assigned to the 50-60 mm class. In order to avoid mixing up the cohorts during measurements, the 40-50 mm class size baskets were tagged green and assigned even numbering (2-18), while the 50-60 mm were tagged blue having odd numbering (1-17) (Appendix A). The baskets (72) were placed into four tanks (523 x 150 x 60 cm) running longitudinally, in the grow-out section of the farm (Fig 1). Shelter in each basket was provided by cylindrical PVC pipes of 21 cm in diameter and 28 cm in height held together with plastic rivets.

Figure 1: Layout showing part of the grow-out section at Marine Growers abalone farm.

Measures of aeration within the grow-out tanks were at an exchange rate of 1 litre per second and a turnover time of approximately 1¼ hours. This was done at the start and end of the study. The ambient water temperature for the duration of this study varied from a maximum temperature of 25.5°C in the summer months to a minimum of 15.0°C in the winter months (Fig 2).

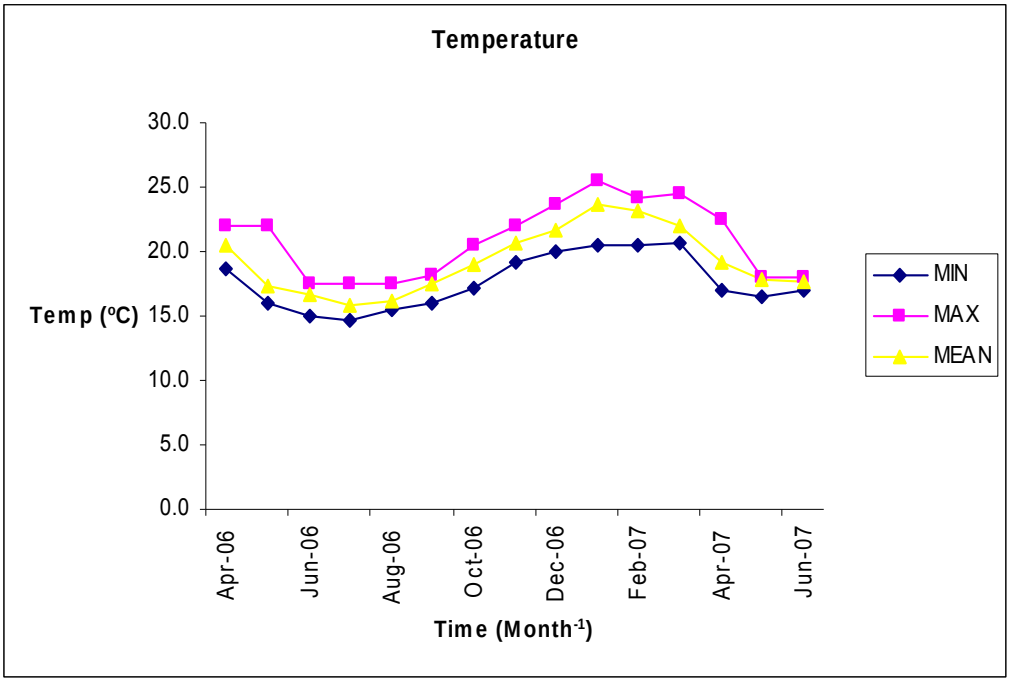

Figure 2: Mean temperature measurements throughout the study.

The 40-50 mm cohort had an initial average shell length of 45.7 ± 1.3 mm and a mass of 20.45 ± 02.33 g; (n = 50) while the length for 50-60 mm cohort was 53.6 ± 1.8mm in length at mass of 29.61 ± 03.19 g; (n =50). The stocking densities for the two class sizes were 758/ m² for the 40-50 mm size class and 455/ m² for the 50-60 mm treatment. Food types fed to the animals were commercial pellets, dried kelp bars and seaweed (*Ulva spp*.). Four diets were obtained from the three food types. These were combination of commercial pellet and seaweed (Diet A), commercial pellet only (Diet B), seaweed only (Diet C) and dried kelp bars only (Diet D). These combinations were made so as to investigate the significance of supplementing the formulated feed with seaweed as opposed to administering

the feed alone (Diet B). Each of the four diets obtained was randomly assigned each to a tank with one of the tanks having the combination of Diet A and C.

Feeding was done daily in accordance with the farm feeding time table, with tank 1 receiving a mixture of commercial pellets and seaweed diet while, tanks 2, 3 and 4 received commercial pellets, seaweed, and dried kelp bars, respectively (Appendix B).

Feed was never left for more than 12 hours with uneaten food removed by hosing the baskets or picking out the feed, prior to adding new feed. This was done so as to maintain water quality. No aspects of the commercial grow-out process were manipulated or interfered with except diet fed (natural lighting was used through out the study and never was artificial lighting used). The feeding was never changed throughout the study period.

After 183 days into the study, the abalone were again sorted into four sizes classes, i.e., 40-50 mm, 50-60 mm, 60-70 mm and 70-90 mm. The animals were sorted into 98 baskets which, were distributed into 8 tanks. The original 40-50 mm cohort in tanks 1, 2, 3 and 4 were sorted into tanks 5, 6, 7 and 8, respectively, while the 50-60 mm original cohort (tanks 1-4) was maintained in tanks 1 to 4. Tanks 1 and 5 (adjacent) received the mixed diet of commercial pellets and seaweed while, tanks 2 and 6, 3 and 7, 4 and 8 received commercial pellets, seaweed, and dried kelp, respectively (Appendix C). Despite the split, diet remained the same following the origin of the animals prior to the split.

Abalone of shell length 80 mm and greater were removed from the study and sold.

2.2 Measuring and weighing animals

Growth was determined by increment in shell length. The measurements not only included length but also shell width, height and abalone live mass (whole body mass). For the length measurements, a Vernier calliper was used to an accuracy of 0.05 mm. A digital electronic balance was used with an accuracy of 0.01 g. Data were collected fortnightly, with 50 individuals randomly measured from each of four baskets per size class, per tank. Due to the variation in size within each cohort as a result of the initial sorting based on visual cue, data obtained for growth in some baskets gave what would seem as “a negative growth” (Fig 5). This would serve to imply that the abalone showed shrinkage at some point during their growth. Due to the hard nature of the abalone shell, this (shrinkage) is not possible which would be explained by measurement of animals with broken shells or as an artefact resulting from the random nature in which the animals were measured i.e. never the same individual. Therefore, the assumption made with regard to growth in this study was that, growth was only measurable if >= 0.

2.3 Growth analysis

Two growth models the Gompertz model (Shepherd & Hearn, 1983) and Von Bertalanffy model (Keesing & Wells, 1989) have been used in aquatic studies to determine growth of aquatic animals over a period of time. Since growth of the abalone in this study was attributed to shell increment, the Von Bertalanffy model was best suited for the analysis of linear growth; $\mathbf{L=L\infty\ \{1-exp\ [-K\ (t-t_o)]\}}$

(McShane *et al*, 1988). Unlike previous studies using the Von Bertalanffy model over much longer periods than in the current study, the Von Bertalanffy model was employed with regard to linearization of the data collected. Despite the duration of this study and the use of Von Bertalanffy model, the aim of this study was not to monitor the trend of growth but rather the performance of a given diet on growth. The assumption made was that linearity in growth of the abalone (*H. midae*) was believed to occur up to 80 mm of shell length. Further study is required to confirm this assumption.

2.4 Statistical analysis

A Regression analysis to determine if any significance existed among the four diet components was carried out for the length data. This was then followed by obtaining growth rates for the growth curves of each of the four diets. In order to determine differences in significance among the slopes, analysis of co-variance (ANCOVA) for the growth rates and the Tukey method was used to perform multiple contrast tests (Zar, 1999). The ANCOVA tests require linear input data hence, the use of shell length linearised data. All statistical comparison was significant at a 95% confidence level ($P < 0.05$).

2.5 Data Transformation

The Von Bertalanffy model requires an ongoing reduction in growth rate (Reaburn & Edwards, 2003). Despite the duration required to observe the asymptote, this study showed the reduction in growth rate with regard to length. In the study by Reaburn & Edwards (2003), the linear model was used and it was found to approximate to the Von Bertalanffy model. Hence the linearization of the

Von Bertalanffy curve using $\mathbf{y = ae^{bx}}$; a and b are variables used to linearise the growth curves. e (natural log) and x is a constant value for t_0 obtained from the equation $\mathbf{L=L\infty\ \{1-exp\ [-K\ (t-t_o)]\}}$.

Chapter 3

RESULTS

Length gain in the 40-50 mm class size was highest for the combination of commercial pellet and seaweed (Diet A; 2.64 mm.month^{-1} ; Table 3.1). Commercial pellet (Diet B) yielded a growth rate of; 2.20 mm.month^{-1}. Among the natural diets, seaweed (Diet C) yielded the highest growth rate in comparison to dried kelp (Diet D); 1.71 mm.month^{-1} and 0.66 mm.month^{-1} respectively (Table 3.1). These results suggest that artificial diets both in combination with natural diets or fed on their own yield better growth than natural diets. Our findings are in agreement with other studies (Britz *et al*. (1994), Serviere-Zaragoza *et al*. (2001) and Viana *et al*. (1993)). Here it was also observed that, formulated diets gave superior growth to natural abalone diets. Growth in relation to whole body mass (WBM) was highest for Diet A (4.19 g.month^{-1}) and lowest for Diet D (0.14 g.month^{-1}) (Table 3.1).

Diet A gave a superior growth rate in terms of WBM and length to Diet B (See Table 3.1). Statistical analysis showed that despite this superior growth in terms of WBM or length, there was no significant difference between the two feeding types (Diet A&B). Diet D gave an inferior growth (0.66 mm.month^{-1}) with regard to length when compared to Diet A and B (Fig 3). Artificial food (Diet B) yielded significantly ($P << 0.05$) higher growth rates in length increment to that of natural food types (Diet C and Diet D; Fig. 4).

Diet	A	B	C	D
Length				
40-50 mm	2.64	2.20	1.71	0.66
50-60 mm	2.78	2.35	1.60	0.50
Mass (WBM)				
40-50 mm	4.19	3.35	1.62	0.14
50-60 mm	6.38	4.96	1.96	0.07
Slopes				
40-50 mm	1.2	1.1	0.7	0.3
50-60 mm	1.3	1.1	0.7	0.2

Table 3.1: Composite growth rates (mm.month^{-1}) and whole body mass (g.month^{-1}) for the two cohorts over a 183-day period. (Values are computed from mean length values at t_1 (fortnight 1) and t_{13} (fortnight 13).

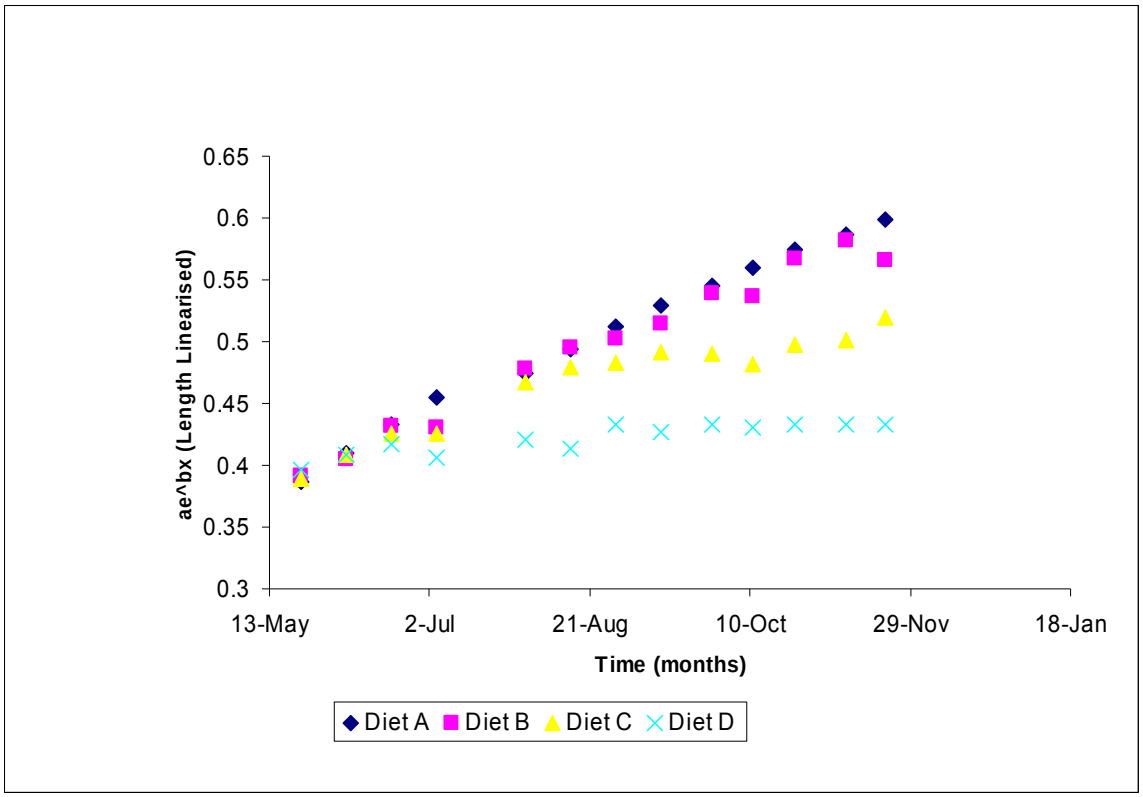

Figure 3: Growth of the 40-50 mm cohort represented per diet over the 183-day period.

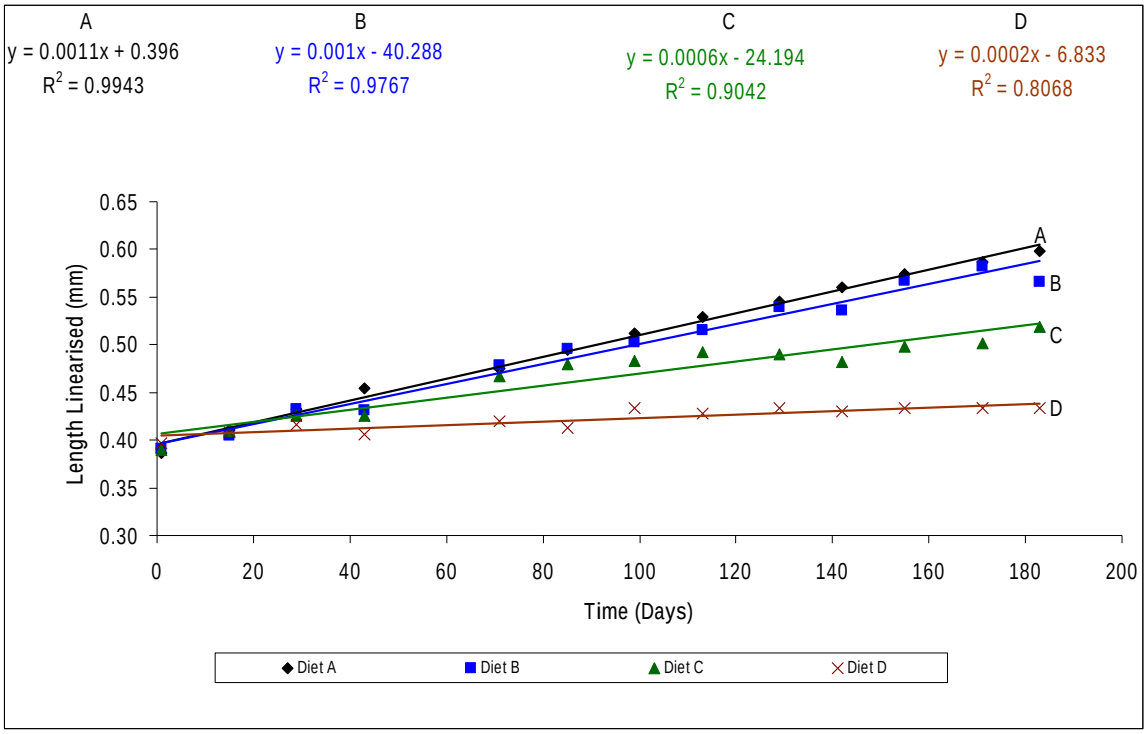

Figure 4: Comparison of growth rates for the 40-50 mm cohort after a 183 day period.

With regard to the natural food types, Diet C gave a significantly ($P < 0.05$) higher growth rate in length than Diet D (Fig 4 & 5). With regard to whole body mass, a similar outcome was observed for the natural when both cohorts were examined.

The 50-60 mm class size had growth rates ranging from 2.78 to 0.50 mm. month^{-1} for the four food components used (Table 3.1). Mass increment was highest for Diet A (commercial pellet and Seaweed) and lowest for Diet D (dried kelp bars); Table 3.1.

The artificial diet (Diet B), yielded a significantly ($P < 0.05$) higher growth rate increment for both length and mass than the natural diets (Diets C and D; Fig 5).

Similar trends in growth between food components as described for the 40-50 mm cohort above were shown by the 50-60 mm cohort (Fig 6).

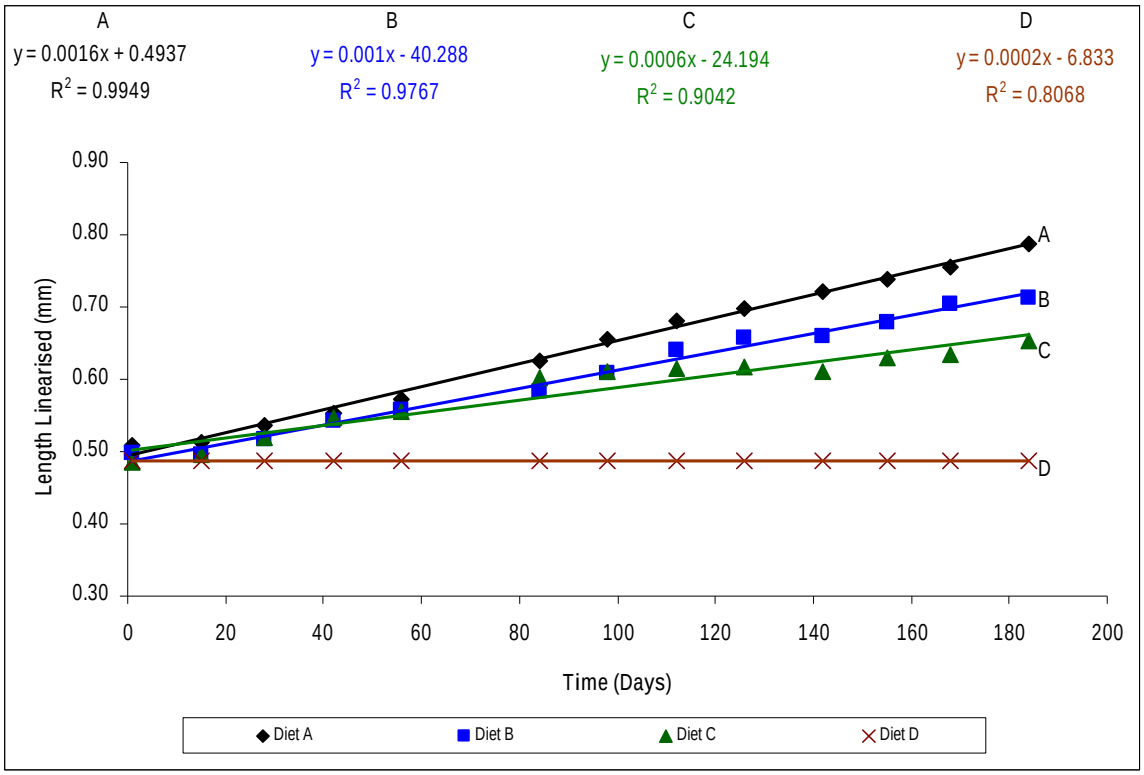

Figure 5: Comparison of growth rates for the 50-60 mm cohort after a 183-day period.

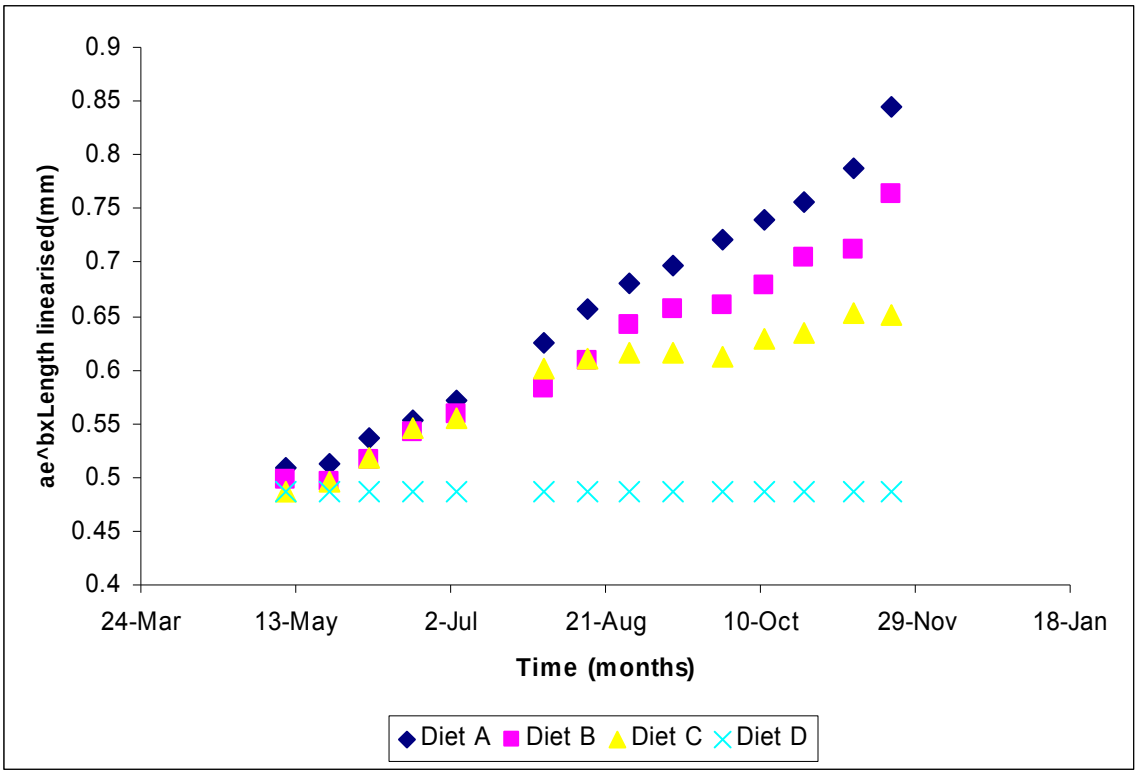

Figure 6 : Growth of the 50-60 mm cohort represented per diet over a 183-day period.

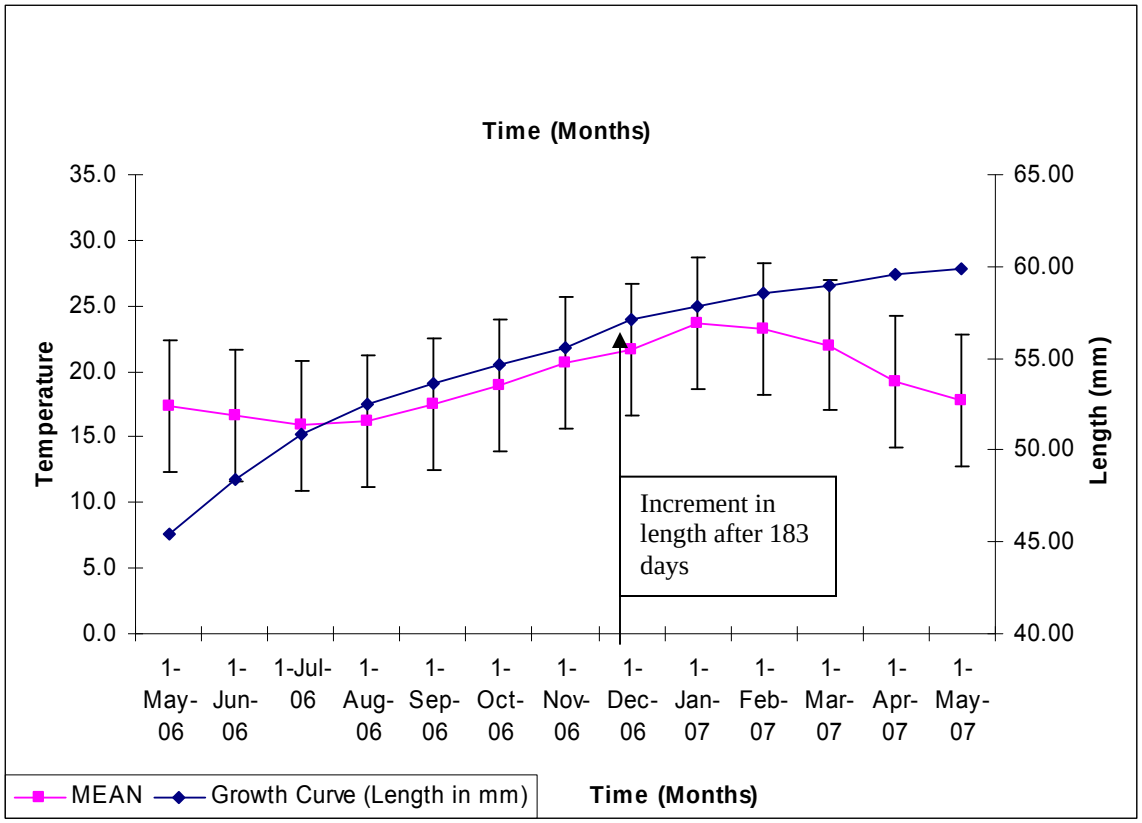

Figure 7: Modelled growth for the 40-50 mm cohort and temperature

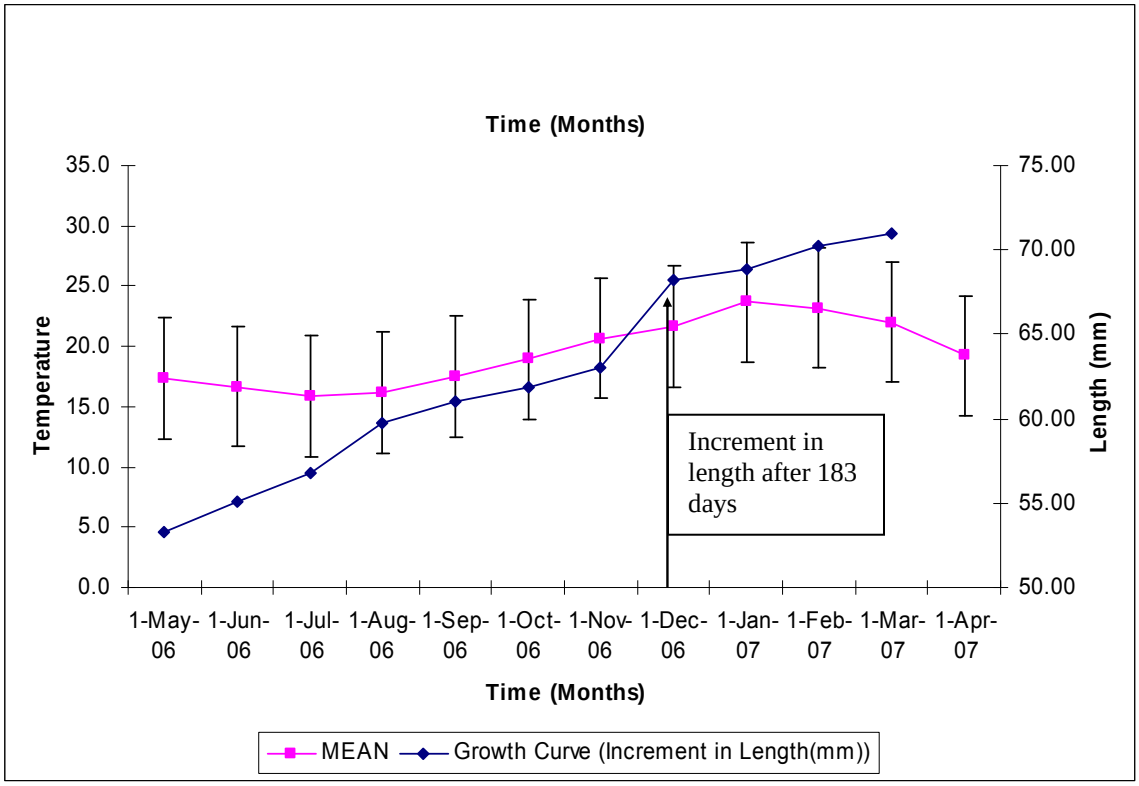

Figure 8: Modelled growth for the 50-60 mm cohort and temperature

Chapter 4

DISCUSSION

The effect of Diet history can influence growth response to test diets (Day & Fleming, 1992; Fleming *et al*. 1996). In order to control for this potentially confounding factor, it has been suggested that preconditioning the animals on a standard diet prior to the feeding trials could minimize the effect of the previous diet (Day & Fleming, 1992). Also serving a similar purpose would be the removal of initial data of approximately 50 days (Day &Fleming, 1992). The assumption I believe is that by these number of days the effect of previous Diet is negligible. In this study the animals prior to being sorted into their size classes were fed a similar diet for more than one year (tanks 1&2 spawned in 2004; tanks 3&4 spawned in 2003). The 183-day period may have played a role in controlling for any effects of previous diet on the growth outcome, although this was not tested in this study. In agreement with the above statement on the duration of the study, studies such as Day &Fleming (1992) and Viana *et al*. (1993) noted that in order to monitor the nutritional effect of a diet, longer trial periods were necessary. Therefore, to a greater extent, the influence on growth we observed for this study can be attributed to the diets fed to abalone.

Four diet components Diet A, B, C & D were used for the feeding trials in this study, with linear increment (length mm) in abalone shell regarded as growth of the animals. Variation with regard to growth rates across the four feed components was evident with time. Numerous studies have pointed to why certain diets out-perform others.

Some of the reasons include; feeding rate on a particular diet (McShane *et al*, 1994) and palatability of diet (Barkai & Griffiths, 1988; Viana *et al*. 1994). These can not be confirmed by the current study since no experimental trial was done with regard to the above reasons for diet out-performance of another.

Nutritional value of the diet may also influence its performance although this did not seem to be the case for *Haliotis rubra* (McShane *et al*, 1994). In the study by Preece & Mladenvor (1999) variation in growth was attributed to low tank water exchange which could compromise the health of the animals following invasion by disease-causing parasites. This would serve to alert management on the importance of keeping clear the water delivery pipes from blockages, which leads to accumulation of waste within tanks and eventually causing disease and death of animals. They also pointed to increased light intensity as another cause for the varied growths. Some of the natural foods utilised by *Haliotis midae* include *Ecklonia maxima*, *Plocamium spp* and *Ulva spp* which is consumed in large quantities by small size abalone (Barkai & Griffiths, 1986). In this study variation in growth attributable to diet was investigated, while other known factors affecting growth (temperature, water flow, stocking density and shape of pellets) were kept constant for all groups.

All Diets showed significance for growth in the p value (=0.0000, with Diet D for 50-60 mm cohort giving p=0.0069). This does not say much about performance of each Diet. As a result, the Analysis of Co-variance was used to test for this performance through the variations observed in growth rate out-come per Diet. Slopes were then compared to test for the null hypothesis $H_o = \beta_1 = \beta_2 = \beta_3 = \beta_4$.

Where β is the population slope (dL/dt = growth rate) of the underlying population.

There was a significant difference ($P<0.05$) between growth rates of Diet D (dried kelp bars) and Diet C (seaweed). In the wild, abalone prefer soft-textured macroalgae (Shepherd & Steinberg, 1992). This may partly explain why in this study, seaweed (*Ulva spp*.) was eaten faster than dried kelp bars (which regularly had a large uneaten proportion prior to the next feeding.) (Personal observation).

Development of a diet either from naturally occurring foods or from artificial food types should focus on efficient utilization of the diet to improve growth of the animal. This efficiency is dependent upon parameters such as palatability (McShane *et al*, 1994; Fleming, 1995; Guzmàn & Viana, 1998; Viana *et al*, 1994), digestibility (Stuart & Brown, 1994; Serviere-Zaragoza *et al*. 2001) and nutritional value (Stuart & Brown, 1994; Serviere-Zaragoza *et al*. 2001) in order to warrant good growth in abalone.

Palatability simply means more of a given food shall be eaten by the organism. This palatability is thought to be caused by presence or absence of attractants (Flemming *et al*, 1996; Viana *et al*, 1994). This may explain why Diet D was the least consumed (visual observation) resulting in low consumption which equates to wastage of feed and eventual low growth rate observed for both cohorts with Diet D. This assumption was not tested for in this study hence further research is required to verify this assumption. In comparison with Diet C (fresh form of diet),

the attractants and nutrition of Diet D could have been lost through the process of "pelletisation".

In the study by de Kock (1997) results obtained from feeding trials on dried kelp bars with two class sizes, yielded growth rates of 1.13 mm.month^{-1}and 0.35 g.month^{-1} for the 20-30 mm cohort, and 1.34 mm.month^{-1} and 0.99 g.month^{-1} for 30-40 mm cohort.

These results are much lower than those obtained in this study as shown in Table 3.1. A plausible reason for the variation could be in the texture of the diet, nutritional content and digestibility of the dried kelp bars (Padilla, 1985; Padilla, 1989; Stuart & Brown, 1994). The higher growth rates shown by the 30-40 mm cohort in comparison to the 20-30 mm (de Kock, 1997) could be that; the latter (cohort) does not easily digest the kelp, owing to the toughness or texture of the diet (Barkai & Griffiths, 1988; Shepherd and Steinberg, 1992; McShane *et al*, 1994). This would mean that the 30-40 mm cohort best utilizes the diet, acquiring the necessary nutrition hence the better growth rate of the two cohorts. The immediate assumption would be that the larger the cohort class size (30-40 mm), the better the growth rate than 20-30 mm. Evidently this is not the case, as is shown by the results (Table 3.1) of this study. A plausible reason could be that as the animals get larger the dried kelp fails to meet their nutritional requirements such as sufficient levels of protein in diet and amino acids. This though was not tested hence we can only speculate this reason.

Most natural abalone food has low protein levels to meet the growth rates as shown by formulated foods. As a result growth over long periods is difficult to maintain at know growth rates as observed in formulated feeds (Britz, 1997). In the study by Stuart & Brown (1994) they conclude by saying, “efficient utilization of a diet is dependent upon the nutritional requirements of the abalone, how well these are matched by the diet and digestibility of the diet. This may be that, dried kelp bars meet the nutritional requirements for the 20-30 mm cohort but the digestibility of this feed is slower than in 30-40 mm cohort; while for the 40- 50 mm and 50-60 mm cohorts, digestibility is quicker but the diet offers a poor nutritional value to sustain the high growth rates, consequently growth slows and seemingly “halts” at some stage (Fig 3).

Day & Fleming (1992), Viana *et al* (1993 & 1996) observed that single species of seaweed fed exclusively to *Haliotis rubra* and *H. fulgens*, respectively, did not sustain growth over extended periods. This is in agreement to the findings of this study with regard to both dried kelp and seaweed (*Ulva spp.*). A plausible explanation could be in the low protein levels (Mai *et al*. 1994 &1996; Hahn 1989) found in seaweed. Given a mixed diet (natural food types), a small but non-significant increase in growth rate was observed in comparison to monospecific diets (Stuart & Brown, 1994). Meaning that the mixed feed gives abalone extra nutritional value hence the greater growth rate observed.

The only artificial food type used in this study was a commercial pellet (10% fishmeal, 5% seaweed powder, 25% soy bean powder, 55% gluten, 2% vitamins and 3% minerals). The 50-60 mm class size gave growth rates of 2.78

mm.month^{-1} for Diet A and 2.35 mm.month^{-1} for Diet B. Despite giving a greater growth rate (Diet A) in comparison to Diet B, there was no statistically significant difference in growth rates between the two diets.

When compared to the growth rates achieved in natural food types both Diet A and B showed a significant difference ($p<0.05$) over Diet C or D. In Britz (1996a) optimal protein levels to warrant good growth rates in *Haliotis midae*, of between 2-3 mm.month^{-1} was put at 47%. On the other hand, Guzman and Viana (1998) state that protein levels of between 36.5%-39.4% are acceptable for good growth rates in abalone.

Artificial diets have been found to elicit a faster feeding response than natural diets (Britz *et al*. 1994). In the study by Britz *et al*. (1994) it was observed that artificial diets were the choice feed when given with natural diets. Since Diet A was the only food component given in combination (artificial & natural foods), the outcome by Britz *et al*. (1994) could not be verified by the current study. Although the possible explanation to a better growth rate for Diet A in comparison to Diet B could be that the seaweed provided with commercial pellets in Diet A, provides certain essential nutritients that may lack or be insufficient in the commercial pellets. The better growth in Diet A is the difference in time taken to grow the animals to marketable size.

Sales & Britz (2002) observed that the reduction of formulated diet particle size to between 150 – 450 µm significantly increased digestibility of the diet in *Haliotis midae*. Therefore, other than factors such as optimal protein levels in diet and

palatability of diet, another plausible reason for the significant variation in growth between the commercial pellet (Diet B) and dried kelp bars (Diet D) would be in the size of pellet. The Diet D pellets were (50x25x5) mm in comparison with the commercial pellets which measured (13x13x4 mm). Therefore, with regard to the study by Sales & Britz (2002), pellet size may have played a role in variation of growth rates observed for this study although, this can only be speculated since no testing was done to ascertain this reasoning.

The effect of environmental temperature on the metabolism of poikilotherms is usually manifested by growth patterns, influencing growth rate as well as maximum size attained (Newman, 1969). Feed consumption in most cultured poikilotherms is primarily determined by temperature and body size (Uki, 1981; Hahn, 1989b, pp, 135-156; Britz1997). Britz *et al*. (1994) argues that if abalone are allowed constant access to feed, the amount consumed is primarily dependent on temperature and body size. They further state that food consumption has been known to more than double between 15°C and 22°C and that the amount ingested expressed as a percentage of body weight decreases with size. This may be consistent with the current study were daily feeding of both cohorts yielded variation in growth rates under similar temperature regimes (Table 3.1; Fig 7&8). Further testing is required to verify this reasoning. As shown by Figure 7 & 8, growth was maintained especially at somewhat cooler temperatures (May-August). The reason for this is that the temperature was within range as stated in the study by Britz *et al* (1994) (15°C and 22°C); hence the animals were actively feeding even amidst winter temperatures.

This study was well in the optimal temperature range for *Haliotis midae* along the Eastern Cape of South Africa. Temperatures between 23°C-25°C were experienced at the farm, these may have attributed to the mortalities experienced at the farm, although this is only speculative.

Chapter 5

CONCLUSION

Despite not having done certain tests, this study has shown similarities with findings of studies such as Britz *et al.* 1994; Bautista-Terrell *et al.* 2003; Capinpin Jr & Core, 1996; Vienna *et al.* 1993 and Nie *et al.* 1986 which, concluded that formulated feeds (both in combination or administered alone) out-perform natural abalone foods with regard to growth achieved. Diet A out-performed all other diets, although the growth rate obtained from this diet showed significance only with regard to Diets C& D. Although no statistical significance was determined between Diet A and B, Diet A showed greater growth which was in agreement with past studies; which observed that *Ecklonia maxima* (Stepto & Cook, (1996)) and *Ulva* (Najmudeen & Victor, 2004; Schoenhoff *et al*, 2003) used as supplements in abalone feeding promoted good growth in *Haliotis midae*.

Diet is not the only factor that affects growth in abalone as has been verified by the studies mentioned above. In order to understand the extent to which each of the following factors will affect growth (temperature, water flow, stocking density and disease), further studies are necessary. In understanding these extents, farm managers can know how best to allocate resources to achieve successful abalone culture. Despite the scope of this study not encompass factors such as temperature, water flow, stocking densities and disease, we still can conclude to a great extent that nutritional value (levels of protein, lipids, carbohydrates and balanced amino acids) of the Diets used played an important role in distinguishing the performance of each diet. Also that, natural abalone diets (for

example; *Ulva, E. maxima*) are still vital to abalone nutritional requirements with best results attained when given as a supplement to diets with adequate protein levels to give growth rates of between 2 -3 mm.month^{-1} (Hahn, 1989) .

The greatest expenditure on many abalone farms globally is the cost of diets both natural and formulated. Most formulated diets today are made from expensive animal protein. As a result of the cost incurred in manufacture of formulated diets, farmers to day are promoting research into a less costly protein food that could give approximately similar growth rates to those given by the more expensive formulated protein feed (between 2 -3 mm.month^{-1} (Hahn, 1989)). Therefore, any diet offering less expensive protein source(s) is one worth investing research into.

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APPENDIX

Appendix A

Basket layout within a tank prior to cohort split from two size classes to four.

17	18
15	16
13	14
11	12
9	10
7	8
5	6
3	4
1 50-60 mm	2 40-50 mm

Appendix B

Tank 1	Commercial pellets and Seaweed (Diet A).
Tank 2	Commercial pellets only (Diet B).
Tank 3	Seaweed only (Diet C)
Tank 4	Dried Kelp bars (Diet D).

Appendix C

Tank layout after the second sorting (split).